life cycle of Dictyostelium and in revealing other nuclear behavior at certain critical points in the life cycle.

* Part of this work was done as a National Research Council Fellow at the University of California, Berkeley.

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A REVERSIBLE PHOTOREACTION CONTROLLING SEED GERMINATION

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Effect of light upon germination of some seed is one manifestation of a very general, possibly universal, phenomenon controlling living processes. Some of the details of this effect on lettuce seed (*Lactuca sativa* L.) are given here. Their bearing on the nature of a *reversible* photoreaction involved is developed.

Germination of one variety of lettuce seed was found by Flint and McAlister[1] and Flint[2] to be promoted by radiation in the region of 5250 to 7000 Å. The greatest response for promotion resulting from a given irradiance was in the region of 6600 Å. Germination was inhibited by radiation in the region of 7000 to 8200 Å. with the maximum inhibition between 7100 and 7500 Å. We have verified the observations of Flint and McAlister for wave-lengths greater than 5200 Å. and have quantitatively determined the action spectrum (Fig. 1).

The action spectrum is expressed in terms of the incident energy required at various wave-lengths to promote or to inhibit germination to half its maximum value. The results were obtained with a two-prism (glass) spectrograph having a dispersion of about 35 Å./cm. at 6000 Å.[3] The slit width was about 90 Å. at this wave-length and the incident energy was 0.100 $\times 10^{-3}$ joules/cm.^{2} sec. Lettuce seeds for these experiments were placed on wetted blotters in dishes that were immediately covered and maintained in darkness at 20°C. for various times before removal for irradiation. Those to be inhibited in germination were first exposed to radiation between 5800 and 6600 Å., as obtained with filters and a 4500° white fluorescent source. The exposure was such as to lead to germination of more than 98% of the viable seeds in controls. Following this treatment the seeds were irradiated in various wave-length regions to test for inhibition of germination. Those to be promoted were taken directly from darkness and irradiated. After irradiation, all seeds were held for two days at 20° in darkness and the ones then germinated were counted.

Two pigments apparently are involved with other reactants in the action of light on germination. Evidence will be given that these materials are directly related by the reactions.

$$\text{(1)} \qquad \underset{\substack{\text{6500 Max.}\\ \text{Dormant}}}{\text{Pigment} + \text{RX}} \quad \overset{\text{Red}}{\rightleftharpoons} \quad \underset{\substack{\text{7300 Max.}\\ \text{Germinating}}}{\text{Pigment X} + \text{R}}$$

$$\text{(2)} \qquad \text{Pigment X} + \text{R} \quad \underset{\substack{\text{Infra Red}\\ \text{Darkness}}}{\rightleftharpoons} \quad \text{Pigment} + \text{RX}$$

$$\text{(3)} \quad \text{with (1)} \quad \text{Sub.} + \text{RX} \longrightarrow \text{R} + \text{Sub. X}$$

$$\text{(4)} \quad \text{with (2)} \quad \text{Sub. X} + \text{R} \longrightarrow \text{RX} + \text{Sub.}$$

Two equations are given since no entirely trustworthy method has been found to associate Pigment and Pigment X with their respective absorption maxima. The nature of the pigment and the possibility of other reactants will not be examined since we do not wish to obscure the immediate deduction of a reversible photoreaction with any speculation.

The sample of Grand Rapids lettuce seed used was equally sensitive to promotion by red radiation for 4 to 16 hr. after imbibition. Fifty per cent germination required 2.11 $\times 10^{-3}$ joules/cm.^{2} of incident energy between 6530 and 6635 Å. and 10.1 $\times 10^{-3}$ joules/cm.^{2} gave 90 per cent germination with a linear variation of the probit with log energy. Seeds that had been exposed to red radiation were equally sensitive to suppression of germination by radiation at wave lengths greater than 7000 Å. over this period of 4 to 16 hr. After 16 hr. of imbibition, the radiant energy required for promotion to a given per cent germination increased and after 31 hr. had changed by about threefold. During this period, the requirement for inhibition decreased by about the same amount. This was the first indication of a relation between the photoreceptive pigments. It probably arises, however, from a thermal conversion of RX or R toward the condition on the right side of the equations.

The dependence of the germination response solely upon the last of a sequence of exposures to red and infra-red radiation is striking evidence of the immediate formation of the one pigment from the other. Seven lots of 200 lettuce seed were exposed to red radiation (5800 to 6800 Å.) for one minute after 16 hr. of imbibition of water in darkness. One lot was removed to the dark and the remaining ones were exposed to infra-red radiation of wave-lengths longer than 7000 Å. for four minutes. Again one lot was removed to the dark and the rest were exposed to red radiation for one minute. The alternations of red and infra-red irradiations were continued until all lots were treated. After this the treated and control lots of seed were held in darkness at 20°C. for two days. The germinated seeds were then counted (table 1).

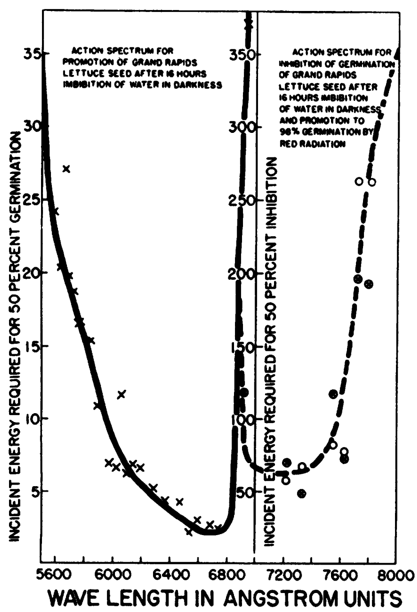

FIGURE 1

The action spectrum for promotion (solid line, ordinate on the left) and inhibition (dotted line, ordinate on the right) for germination of Grand Rapids lettuce seed. The several symbols give results from different experiments. Ordinates are in joules $\times$ $10^{-3}/cm^2$.

Energy greatly in excess of that required for full germination does not appreciably influence the sensitivity to inhibition by infra-red radiation. Thus a thirty-two fold increase in irradiance between 5800 and 6800 Å. over that required for 98 per cent germination of seed soaked for 16 hr. raised by less than twofold the energy required in the infra-red for suppressing the germination to a particular value. The result is in harmony with displacement of the equilibrium in a photoreaction and indicates that the pigment does not serve merely to transfer energy. Rather a certain energy is absorbed to activate the change of the pigment from one form to another.

The increased energy required for a given per cent germination, as the time of imbibition prior to irradiation is prolonged to periods greater than 16 hr., indicates that RX in equation (1) or R in equation (2) is limiting. In other words after long periods of imbibition, energy absorbed by the pigment is dissipated in some way, such as fluorescence, before reaction (1) or (2) takes place. That the pigment or pigment X is not limiting is shown by the simultaneous decrease in the energy required for inhibition. In fact this change in response is one evidence that a reactant other than the pigment is involved in the photoreaction.

After displacement by radiation does the reaction return toward an equilibrium in darkness? Experiments to answer this question must avoid confounding the sequence of germination following the displacement of the reaction to the right with a possible back reaction having about the same time constant. Seed of Grand Rapids lettuce was held fully imbibed at 20°C. for 16 hr. and then irradiated with energy adequate for full promotion of germination. This treatment was followed 7 hr. later by irradiation in the infra-red adequate to inhibit germination fully when given immediately after promotion. About 50 per cent of the seeds germinated. This indicates that in 7 hr. the germination process in half the promoted seeds had proceeded beyond determination by light.

An answer to the question was afforded by germination of a white-seeded lettuce variety that was thought to be more sensitive to temperature than Grand Rapids. Seeds of this variety germinate in light or in darkness at 20°C. but are dormant at 30°. After having been held for several days in

TABLE 1

LETTUCE SEED GERMINATING IN POPULATIONS OF 200 AFTER EXPOSURE TO RED AND INFRA-RED (IR) RADIATION IN SEQUENCE

IRRADIATION	GERMINATION, %
None (dark control)	8.5
Red	98
Red + IR	54
Red + IR + Red	100
Red + IR + Red + IR	43
Red + IR + Red + IR + Red	99
Red + IR + Red + IR + Red + IR	54
Red + IR + Red + IR + Red + IR + Red	98

darkness at 30° these seeds remain dormant if returned to 20° in darkness. However, if they are first irradiated in the region 5800 to 6800 Å. before returning to 20° in darkness, full germination is promoted. It follows that the reaction for the pigment was displaced from right to left in darkness at 30°. Existence of such a dark reaction indicates that the radiant energy is chiefly required for pigment activation rather than for the free energy change in the reaction.

The reaction can be poised at any point for a time adequate for germination; that is, a photochemical steady state can be established. The per cent of germination depends upon the integrated absorption of the two pigments. Thus in some wave band between 6900 and 7100 Å., 50 per cent germination is effected by continuous irradiation.

We have not thoroughly studied the action spectrum in the region below 5200 Å. An irradiance of 90×10^{-3} joules/cm.^{2} in the region of 4600 Å. resulted in about 60 per cent germination of Grand Rapids lettuce seed that had been held imbibed for 16 hr. in darkness. Less than 0.75×10^{-3}

joules/cm.[2] gave 50 per cent germination. This indicates that blue radiation can be very effective for promotion of germination. Failure of promotion to full germination as well as absence of an inhibitory action of blue radiation on seed that had been irradiated in the red adequate for full germination requires more detailed study for understanding.

The immediate general extension of these observations is to the varied response of seed germination to radiation.[4,5] Germination of some seed is promoted by continuous light in the visible,[6] while that of others such as *Phacelia tanacetifolia* Benth. and *Amaranthus caudatus* L. is inhibited.[7] This is a result of the integrated effect of the photoreaction and suggests that the amount of the pigments and reactants R and RX vary among seeds. Many seeds that apparently are insensitive to control of germination by light nevertheless have the reaction for control. Thus the white-seeded lettuce variety previously mentioned germinates under radiation from an incandescent-filament lamp or in darkness but fails when exposed to radiation having wave-lengths in the region of 7000 to 8000 Å.

Dormancy in a seed is a suspended animation for the small plant that is present. Dormant imbibed seeds are known to have been viable for at least 70 years.[8] In these cases the time is so great as to raise serious question about maintenance of organization for future germination by slow but continued expenditure of energy in respiration. Imbibed seeds that have been held for long periods in darkness germinated after they were finally examined in light. If, following examination, the seeds had been irradiated in the region from 7000 to 8000 Å, the dormancy would likely have been reestablished. This, for seeds, is the control of the living process that was mentioned in the first paragraph.

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